Center for Veterinary Biologics
and
National Veterinary Services Laboratories
Testing Protocol

Supplemental Assay Method for Potency of Live Avirulent *Pasteurella haemolytica* Vaccine

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1. Introduction

1.1 Background

This Supplemental Assay Method (SAM) establishes the titration method for the analysis of avirulent *Pasteurella haemolytica* vaccine, to determine the colony-forming units (CFU) in final container samples, as prescribed by the Code of Federal Regulations, Title 9 (9 CFR), Part 113.68(c)(2). This method uses Tryptic Soy Broth (TSB) as a diluent and Tryptic Soy Agar (TSA) plates with 5% sheep blood for determining CFUs.

1.2 Keywords

Pasteurella haemolytica, potency test

2. Materials

2.1 Equipment/instrumentation

2.1.1 Vortex mixer

2.1.2 Colony counter

2.1.3 Inoculum spreader

2.1.4 Bunsen burner

2.1.5 Disposable syringes and needles--appropriate sizes

2.1.6 Sterile disposable pipettes--appropriate sizes

2.1.7 Sterile screw-capped (sc) culture tubes

2.1.8 Pipetting aid

2.1.9 37° $\pm$ 2°C incubator

2.1.10 Biosafety cabinet

2.1.11 Gloves and lab coat or frock

2.1.12 4 x 4-in sterile gauze pads

2.1.13 Test tube rack

2.2 Reagents/supplies

2.2.1 Trypticase Soy Broth (TSB) (**Section 9.1.1**) National Veterinary Services Laboratories (NVSL) Media No. 10404.

2.2.2 Trypticase Soy Agar with 5% Sheep Blood (**Section 9.1.2**) NVSL Media No. 10218 or as stated in the Outline of Production (OP) from the biologics manufacturer

2.2.3 *P. haemolytica* reference culture (American Type Culture Collection [ATCC] #33396)

2.2.4 70% ethyl alcohol

2.2.5 Sterile water in serum vials--volumes determined by referring to the biologics manufacturer's OP or as stated on the vaccine vial

3. Preparation for the test

3.1 Personnel qualifications/training

The personnel performing the test must have experience or training in this SAM. This includes knowledge of aseptic biological laboratory techniques and preparation, proper handling, and disposal of biological agents, reagents, tissue culture samples, and chemicals. The personnel must also have knowledge of safe operating procedures and policies and Quality Assurance (QA) guidelines of the CVB-L or equivalent, as well as training in the operation of the necessary laboratory equipment listed in **Section 2.1**.

3.2 Preparation of equipment/instrumentation

3.2.1 Turn on the biosafety cabinet 60 min before use and turn off after use. Wipe down the interior of the hood with 70% ethyl alcohol before and after use.

3.2.2 Monitor incubators daily for temperature according to the current version of GDOCSOP0001.

3.2.3 Monitor freezers and coolers used for storing samples daily for temperature according to the current version of GDOCSOP0003.

3.3 Preparation of reagents/control procedures

3.3.1 Warm the samples and reference culture to room temperature before rehydrating to the appropriate volume.

3.3.2 Prepare the *P. haemolytica* reference control samples according to the current version of STRPP0001.

3.3.3 Negative and Positive Controls: Incubate 2 uninoculated plates of TSA with 5% sheep blood with test sample plates as negative control plates. Dilute the *P. haemolytica* reference culture (positive control) the same as the test samples and plate depending on the titer found in **Section 3.3.2.**

3.3.4 Store plates used for making counts at refrigerator temperature. Place plates to be used for counts in a 35° ± 2°C incubator overnight prior to use or allow to dry in a biosafety cabinet before use. At the time of use, plates are no more than 14 days old.

3.4 Preparation of the sample

Samples of *P. haemolytica* vaccines and/or combination products containing this fraction are received from the Biological Materials Processing Section (BMPS) according to the current version of STSOP0001.

4. Performance of the test

4.1 Number all tubes and plates with the sample number, the vial number, and the dilution.

4.2 Disinfect 2 vials of vaccine with 70% alcohol and flame the tops lightly.

4.3 Rehydrate desiccated products with the required amounts of sterile diluent or sterile water if diluent is not supplied, using a syringe and needle. Allow sufficient time for rehydration. Mix the vials by shaking until lyophilized cake is completely dissolved.

4.4 Remove the caps from the vials of vaccine with a decapper.

4.5 Transfer 1 ml of vaccine from each vial into 2 tubes containing 9 ml TSB each (this is the 10^{-1} dilution).

4.6 Shake the 10^{-1} dilution tubes on a vortex mixer. Transfer 1 ml of the 10^{-1} suspension (using a pipette) to tubes with 9 ml TSB (10^{-2}) and mix on a vortex mixer. Continue this dilution series through the 10^{-6} dilution. Transfer 0.1 ml (using a pipette) of the $l0^{-4}$, $l0^{-5}$, and 10^{-6} dilution to each of the 3 TSA plates with 5% sheep blood per dilution. Spread the inoculum over the entire surface of the medium with a sterile spreader.

4.7 Incubate the plates in an inverted position at 35° $\pm$ 2°C for 24 hr.

4.8 Count the colonies on the plates of the dilution having 30-300 CFU per plate. Average the colony count per vial and then for the 2 vials.

5. Interpretation of the test results

5.1 If on the initial test the CFU per dose is equal to or exceeds the required minimum as written in the firm's OP, the serial or subserial is satisfactory (SAT) for bacterial count without additional testing.

5.2 If on the initial test the CFU per dose is less than the required minimum as written in the firm's OP, the serial or subserial may be retested using 4 new vaccine samples, provided that if the retest (RT) is not done, the serial or subserial is unsatisfactory (UNSAT). Compare the firm's OP method to this SAM method when retesting the 4 vials. If on the RT, the average count of the 4 vaccine samples with the firm's OP method is less than the required minimum, the serial or subserial is UNSAT.

5.3 If on the RT with 4 vials, the average using the firm's OP method count is equal to or exceeds the required minimum, the serial is SAT.

5.4 If on the initial test the reference culture or positive control culture is not within the titer range determined in **Section 3.3.2**, but the serial being tested has a SAT result, the serial or subserial is a no test (NT) for bacterial count without additional testing, and the product is released on the results of the firm's tests. If the reference culture is not within its titer range and the serial being tested is below its minimum release titer, the serial is retested using 2 new vaccine samples. If on the initial test there is growth on the negative control plates, the serial or subserial is a NT for bacterial count without additional testing.

6. Report of test results

6.1 Record the CFU per dose along with the final conclusion for the product tested on the log book record sheet (STFRM00PT) and the computer worksheet after calculating the CFU per dose and interpreting the results. Enter the results and conclusions into the computer under the ST test code 068-PT2 as stated in the current version of STSOP0021.

6.2 Initial and date the log book record sheet and the computer worksheet. Forward all paperwork to the CY/ST supervisor or microbiologist to review and sign.

6.3 Validate the test results according to the current version of STSOP0021 and file all paperwork appropriately.

7. Reference

Code of Federal Regulations, Title 9, Part 113.68(c)(2), U.S. Government Printing Office, Washington, DC, 1999.

8. Summary of revisions

This document was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use in the CVB-L, and to provide additional detail. No significant changes were made from the previous protocol.

9. Appendices

9.1 NVSL Media Formulation No. 10423

Trypticase Soy Broth (TSB)
or
Soybean Casein Digest Medium (SCDM)

Trypticase Soy Broth	30 g
QH_2O	1000 ml

Autoclave 20 min at 121°C. TSB and SCDM are 2 names for the same media formulation from different media companies.

9.2 NVSL Media Formulation No. 10210

Trypticase Soy Agar (TSA) with 5% Sheep Blood

Trypticase Soy Agar	40 g
QH_2O	950 ml

Mix and autoclave 20 min at 121°C. Cool at 56°C in a waterbath. Add 50 ml defibrinated sheep blood.